Molecular Biology

FUNCTIONAL INTERACTIONS IN THE RIBOSOMAL DECODING REGION

Laurie A. Boore, Ashesh A. Saraiya, Marny W. Waddington,
and Philip R. Cunningham*
prometheus1717@yahoo.com
Wayne State University
Dept. of Biological Sciences
5047 Gullen Mall
Detroit MI 48202

Ribosomes are macromolecular machines composed of RNA and protein that catalyze protein synthesis in all organisms. The fundamental processes involved in translation are believed to be the same in all organisms and it is now clear that ribosomal RNA plays a direct catalytic role in this process. One of the most conserved sequences of rRNA is the ribosomal decoding region, which is the site of codon-anticodon recognition and is targeted by a number of antibiotics. Previous studies have shown that altering the base sequence in the decoding region dramatically affects protein synthesis and is frequently lethal.

To examine the molecular interactions in the decoding region, a genetic system was developed in the Cunningham Lab to study rRNA without interfering with normal cellular function. In this system, normally deleterious rRNA mutations in plasmid encoded ribosomes only inhibit translation of chloramphenicol acetyltransferase (CAT) and green fluorescent protein (GFP) mRNAs. This system allows rapid and accurate analysis of ribosome function. Four conserved nucleotides in the decoding region, two of which are modified nucleotides, have been shown to play vital roles in ribosome function: A1500 and m4Cm1402 are proposed to form a non-Watson-Crick base pair and mutations at G1505 have been proposed to suppress a normally lethal m5C1407U mutation. To examine the potential involvement of these nucleotides in protein synthesis, single mutations were constructed at each position, double mutants were constructed for each pair of nucleotides, and the effect of each mutation was determined.

The results show that C1402/A1500 mutants with the highest function had purines at position 1500 and pyrimidines at position 1402. These results, coupled with the recent publication of the 30 S ribosomal RNA crystal structure in *Nature*, suggest that the identity of these two nucleotides is important for ribosome activity. Single mutations at position 1505 and at position 1407 blocked protein synthesis. Double mutants between positions 1505 and 1407U were also inactive. These findings indicate that the conservation of the wild type nucleotides is important for proper ribosome function. Construction of 1505 and 1407 double mutants is currently underway. Genetic complementation and

biochemical characterization will be used to identify the mechanism of loss of function.